

CLAIMS

1. A production method of a mammalian artificial chromosome, comprising:
 - 5 a first step of introducing a first vector being circular in form and comprising a mammalian centromere sequence and a second vector being circular in form and comprising a functional sequence into a mammalian host cell;
 - a second step of selecting transformed cells; and
 - 10 a third step of selecting a cell containing a mammalian artificial chromosome from the selected transformed cells.
2. A production method of a mammalian artificial chromosome, comprising:
 - 15 a first step of introducing a first vector consisting of a yeast artificial chromosome having a mammalian centromere sequence and a mammalian telomere sequence and a second vector consisting of a yeast artificial chromosome having a functional sequence into a mammalian host cell;
 - a second step of selecting transformed cells; and
 - 20 a third step of selecting a cell containing a mammalian artificial chromosome from the selected transformed cells.
3. The production method according to claim 1 or 2, wherein the first vector has a selection marker gene and the selection of the transformed cells in
25 the second step is carried out by using the selection marker gene.
4. The production method according to any of claims 1 to 3, wherein the mammalian centromere sequence comprises a region in which a plurality of the following sequences are arranged at regular intervals:
 - 30 5'-NTTCGNNNNANNCGGGN-3': SEQ ID NO. 1, wherein N is selected from the group consisting of A, T, C and G.
5. The production method according to any of claims 1 to 4, wherein the mammalian centromere sequence comprises a sequence derived from a human
35 chromosome alpha satellite region.

6. The production method according to claim 5, wherein the mammalian centromere sequence comprises a 11mer repeat unit derived from a human chromosome 21.
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7. The production method according to any of claims 1 to 6, wherein the size of the mammalian centromere sequence is about 50 kb or less.
8. The production method according to any of claims 1 to 7, wherein the functional sequence consists of a sequence encoding a target gene and a regulatory region thereof.
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9. The production method according to claim 8, wherein the target gene is a gene other than housekeeping genes.
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10. The production method according to claim 8, wherein the target gene is a structural gene of human guanosine triphosphate cyclohydrolase I.
11. The production method according to claim 8, wherein the functional sequence is a sequence encoding an entire region of a human β globin gene cluster.
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12. The production method according to any of claims 1 to 7, wherein the functional sequence consists of an insertion sequence for specifically inserting a sequence of interest.
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13. The production method according to claim 12, wherein the insertion sequence is a loxP site, a FRT site, or a sequence obtained by partial modification of a loxP site or a FRT site and has a function for inserting the sequence of interest.
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14. The production method according to any of claims 1 to 13, wherein the quantity ratio of the first vector to the second vector, which are inserted in the first step, is in the range from about 10 : 1 molecular ratio to about 1 : 10 molecular ratio.
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15. The production method according to any of claims 1 to 14, wherein a plurality of vectors comprising different functional sequences are used as the second vector.
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16. The production method according to any of claims 1 to 15, wherein the second vector further comprises an insulator sequence.
17. A mammalian artificial chromosome obtainable by the production method described in any of claims 1 to 16,
- 10 which comprises a mammalian replication origin, a mammalian centromere sequence and a functional sequence; and
- which is circular in form and is replicated in a mammalian cell, maintained extrachromosomally in a host cell, and transmitted to daughter cells
- 15 during cell division.
18. A mammalian artificial chromosome obtainable by the production method described in any of claims 1 to 16,
- which comprises a mammalian replication origin, a mammalian
- 20 centromere sequence, a mammalian telomere sequence, and a functional sequence encoding a target gene and a regulatory region thereof; and
- which is linear in form and is replicated in a mammalian cell, maintained extrachromosomally in a host cell, and transmitted to daughter cells
- 25 during cell division.
19. A mammalian artificial chromosome,
- which comprises a mammalian replication origin, a mammalian centromere sequence, and a functional sequence encoding a target gene (excluding a housekeeping gene) and a regulatory region thereof, and
- 30 which is circular in form and is replicated in a mammalian cell, maintained extrachromosomally in a host cell, and transmitted to daughter cells during cell division.
20. The mammalian artificial chromosome according to claim 19, wherein
- 35 the target gene is a structural gene of a human guanosine triphosphate

cyclohydrolase I.

21. A mammalian artificial chromosome,
which comprises a mammalian replication origin, a mammalian
5 centromere sequence, a mammalian telomere sequence, and a functional
sequence encoding a target gene (excluding a housekeeping gene) and a
regulatory region thereof, and
which is linear in form and is replicated in a mammalian cell,
maintained extrachromosomally in a host cell, and transmitted to daughter cells
10 during cell division.
22. The mammalian artificial chromosome according to claim 21, wherein
the functional sequence consists of an entire region of a human β globin gene
cluster.
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23. A mammalian artificial chromosome,
which comprises a mammalian replication origin, a mammalian
centromere sequence, and an insertion sequence for specifically inserting a
sequence of interest, and
20 which is circular in form and is replicated in a mammalian cell,
maintained extrachromosomally in a host cell, and transmitted to daughter cells
during cell division.
24. A mammalian artificial chromosome,
25 which comprises a mammalian replication origin, a mammalian
centromere sequence, a mammalian telomere sequence, and an insertion
sequence for specifically inserting a sequence of interest,
which is linear in form and is replicated in a mammalian cell,
maintained extrachromosomally in a host cell, and transmitted to daughter cells
30 during cell division.
25. The mammalian artificial chromosome according to claim 23 or 24,
wherein the insertion sequence is a loxP site, a FRT site, or a sequence obtained
by partial modification of a loxP site or a FRT site and has a function for
35 inserting the sequence of interest.

26. The mammalian artificial chromosome according to any of claims 17 to 25, wherein the mammalian centromere sequence comprises a region in which a plurality of the following sequences are arranged at regular intervals:

5 5'-NTTCGNNNNANNCGGGN-3': SEQ ID NO. 1, wherein N is selected from the group consisting of A, T, C and G.

27. The mammalian artificial chromosome according to any of claims 17 to 25, wherein the mammalian centromere sequence comprises a sequence derived
10 from a human chromosome alpha satellite region.

28. The mammalian artificial chromosome according to claim 27, wherein the mammalian centromere sequence comprises an 11mer repeat unit derived
15 from a human chromosome 21.

29. The mammalian artificial chromosome according to any of claims 17 to 28, comprising a plurality of the functional sequences or the insertion sequences.

20 30. The mammalian artificial chromosome according to any of claims 17 to 29, further comprising an insulator sequence.

31. A mammalian cell containing the mammalian artificial chromosome described in any of claims 17 to 30 outside the autonomous chromosome.
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32. A human cell containing the mammalian artificial chromosome described in any of claims 17 to 30 outside the autonomous chromosome.

33. An embryonic stem cell containing the mammalian artificial
30 chromosome described in any of claims 17 to 30 outside the autonomous chromosome.

34. A production method of a mammalian cell in which the functional sequence or the insertion sequence is introduced in a state in which they can be
35 maintained stably for a long term, the method comprising:

introducing the mammalian artificial chromosome obtained by the production method described in any of claims 1 to 16 or the mammalian artificial chromosome described in any of claims 17 to 30 into mammalian cells as target cells.

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35. A production method of a mammalian cell containing a mammalian artificial chromosome, the method comprising:

10 a first step of introducing a first vector being circular in form and comprising a mammalian centromere sequence and a second vector being circular in form and comprising a functional sequence into mammalian host cells;

a second step of selecting transformed cells;

a third step of selecting a cell containing a mammalian artificial chromosome from the selected transformed cells;

15 a fourth step of isolating the mammalian artificial chromosome from the selected cells; and

a fifth step of introducing the isolated mammalian artificial chromosome into a mammalian cell as a target cell.

20 36. A production method of a mammalian cell containing a mammalian artificial chromosome, the method comprising:

a first step of introducing a first vector consisting of a yeast artificial chromosome having a mammalian centromere sequence and a mammalian telomere sequence and a second vector consisting of a yeast artificial chromosome having a functional sequence into mammalian host cells;

25 a second step of selecting transformed cells;

a third step of selecting a cell containing a mammalian artificial chromosome from the selected transformed cells;

30 a fourth step of isolating the mammalian artificial chromosome from the selected cell; and

a fifth step of introducing the isolated mammalian artificial chromosome into a mammalian cell as a target cell.

37. A production method of a micro-cell containing a mammalian artificial chromosome, the method comprising:

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- a first step of introducing a first vector being circular in form and comprising a mammalian centromere sequence and a second vector being circular in form and comprising a functional sequence into mammalian host cells;
- 5 a second step of selecting transformed cells;
- a third step of selecting a cell containing a mammalian artificial chromosome from the selected transformed cells;
- a fourth step of fusing the selected cell with a mammalian cell having an ability of forming micro-cells;
- 10 a fifth step of selecting a hybrid cell capable of forming micro-cells and containing the mammalian artificial chromosome; and
- a sixth step of forming micro-cells from the selected hybrid cell.
38. A production method of a micro-cell containing a mammalian artificial chromosome, the method comprising:
- 15 a first step of introducing a first vector consisting of a yeast artificial chromosome including a mammalian centromere sequence and a mammalian telomere sequence and a second vector consisting of a yeast artificial chromosome including a functional sequence into mammalian host cells;
- 20 a second step of selecting transformed cells;
- a third step of selecting a cell containing a mammalian artificial chromosome from the selected transformed cells;
- a fourth step of fusing the selected cell with a mammalian cell having an ability of forming micro-cells;
- 25 a fifth step of selecting a hybrid cell having an ability of forming micro-cells and containing a mammalian artificial chromosome; and
- a sixth step of forming micro-cells from the selected hybrid cell.
39. A production method of mammalian cells containing a mammalian artificial chromosome, comprising:
- 30 fusing the micro-cell obtainable by the production method described in claim 37 or 38 with a mammalian cell as a target cell.
40. A production method of a mammalian cell containing a mammalian artificial chromosome, comprising:
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isolating the mammalian artificial chromosome from the host cell containing the mammalian artificial chromosome described in any of claims 17 to 30; and

5 introducing the isolated mammalian artificial chromosome into a mammalian cell as a target cell.

41. A production method of a micro-cell containing a mammalian artificial chromosome, the method comprising:

10 fusing a host cell containing the mammalian artificial chromosome described in any of claims 17 to 30 and a mammalian cell having an ability of forming micro-cells;

selecting a hybrid cell having an ability of forming micro-cells and containing the mammalian artificial chromosome; and

15 forming micro-cells from the selected hybrid cells.

42. A production method of a mammalian cell containing a mammalian artificial chromosome, the method comprising:

20 fusing the micro-cell obtainable by the production method described in claim 41 with a mammalian cell as a target.

43. The production method of a mammalian cell according to any of claims 34, 35, 36, 39, 40 and 42, wherein the mammalian cell as a target cell is an embryonic stem cell, embryonic germ cell, or tissue stem cell.

25 44. The production method of a mammalian cell according to any of claims 34, 35, 36, 39, 40 and 42, wherein the mammalian cell as a target cell is formed by inducing an embryonic stem cell, embryonic germ cell, or tissue stem cell so as to be differentiated to a cell of specific tissue.

30 45. The production method of a mammalian cell according to any of claims 34, 35, 36, 39, 40 and 42, wherein the mammalian cell as a target cell is a fertilized egg.

35 46. A vector used for producing a mammalian artificial chromosome, comprising a mammalian centromere sequence having the size of about 50 kb or

less and a selection marker gene.

47. The vector according to claim 46, wherein the mammalian centromere sequence comprises a region in which a plurality of the following sequences are arranged at regular intervals:

5'-NTTCGNNNNANNCGGGN-3': SEQ ID NO. 1, wherein N is selected from the group consisting of A, T, C and G.

48. The vector according to claim 46 or 47, wherein the mammalian centromere sequence comprises a sequence derived from a human chromosome alpha satellite region.

49. The vector according to claim 48, wherein the mammalian centromere sequence comprises an 11mer repeat unit derived from a human chromosome 21.

50. A vector used for producing a mammalian artificial chromosome, comprising: a sequence of a loxP site or FRT site, or a sequence obtainable by partial modification of a loxP site or FRT site, the sequence having a function for inserting the sequence of interest, and an insulator sequence.

51. A non-human transformed animal into which a mammalian artificial chromosome is introduced.

52. The non-human transformed animal according to claim 51, wherein the mammalian artificial chromosome is a mammalian artificial chromosome described in any of claims 17 to 19.

53. An XO type mouse embryonic stem cell into which a mammalian artificial chromosome is introduced.

54. The XO type mouse embryonic stem cell according to claim 53, wherein the mammalian artificial chromosome is a mammalian artificial chromosome described in any of claims 17 to 19.

55. A female chimeric mouse into which a mammalian artificial chromosome is introduced.

- 5 56. The female chimeric mouse according to claim 55, wherein the mammalian artificial chromosome is a mammalian artificial chromosome described in any of claims 17 to 19.